

WHAT IS CLAIMED IS:

1. A high-affinity mouse monoclonal antibody to human tumor necrosis factor- α (TNF α), wherein said monoclonal antibody (a) competitively inhibits the binding
5 of antibody A2 to TNF and (b) binds to a neutralizing epitope of human TNF α .

2. A mouse monoclonal antibody according to claim 1 in detectably labeled form.

3. A chimeric immunoglobulin chain comprising
10 at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region having specificity to human TNF α .

4. A chimeric immunoglobulin chain according to claim 3, wherein said chain is a heavy chain or a light
15 chain.

5. A chimeric immunoglobulin chain according to claim 3, wherein said constant region is of human origin.

6. A chimeric antibody molecule comprising two light chains and two heavy chains, each of said chains
20 comprising at least part of a constant region and at least part of a variable region, said variable region having specificity to human TNF α , said antibody binding with high affinity to a neutralizing epitope of human TNF α *in vivo*.

7. A chimeric antibody according to claim 6
25 which does not bind to TNF β .

8. A chimeric antibody according to claim 6, wherein said variable or constant region is of murine origin.

5 9. A chimeric antibody according to claim 8, wherein said variable region is derived from a high affinity murine monoclonal antibody which binds to a neutralizing epitope of human $\text{TNF}\alpha$.

10 10. A chimeric antibody according to claim 9, wherein said murine monoclonal antibody competitively inhibits the binding of A2 or cA2 to $\text{TNF}\alpha$.

11. A chimeric antibody according to claim 9, wherein said murine monoclonal antibody is A2.

15 12. A chimeric antibody according to claim 6 wherein said affinity, measured as an association constant (K_a), is at least 1×10^8 liter/mole. *112-1 infinity*

13. A chimeric antibody according to claim 6 wherein said affinity, measured as an association constant (K_a), is at least 1×10^9 liter/mole. *v*

20 14. A chimeric antibody according to claim 6 which neutralizes human $\text{TNF}\alpha$ with an ID_{50} of at least about $1 \mu\text{g/ml}$.

15. A chimeric antibody according to claim 6 which neutralizes human $\text{TNF}\alpha$ with an ID_{50} of at least about 100 ng/ml .

25 16. A chimeric antibody according to claim 6 which neutralizes human $\text{TNF}\alpha$ with an ID_{50} of at least about 15 ng/ml .

17. A chimeric antibody according to claim 6 in detectably labeled form.

18. A monoclonal antibody according to claim 1 in detectably labeled form which is produced by a hybridoma or recombinantly.

19. A monoclonal antibody according to claim 1, wherein said antibody has an antigen binding region which binds residues 87-108, or both 59-80 and 87-108, of hTNF α of SEQ ID NO:1.

20. An antibody according to claim 1, wherein said antibody, fragment or region does not bind to one or more epitopes included in amino acids 11-13, 37-42, 49-57 or 155-157 of hTNF α of SEQ ID NO:1.

21. An anti-TNF antibody, or a fragment or region thereof, having an anti-TNF binding region, or fragment thereof, corresponding to a

(a) murine monoclonal antibody of monoclonal antibody A2; or

(b) chimeric mouse-human monoclonal antibody, fragment or region of monoclonal antibody cA2.

22. A TNF peptide comprising at least 5 amino acids selected from the group consisting of amino acids residues 87-108 or both residues 59-80 and 87-108 of hTNF α of SEQ ID NO:1, wherein said peptide comprises an epitope of an anti-TNF antibody, fragment or region having anti-TNF biological activity by binding to a TNF sequence other than a receptor binding locus, such that TNF binding to a TNF receptor is substantially inhibited.

23. A TNF peptide according to Claim 22, consisting essentially of 3 to 22 amino acids of at least one of the sequences

5 Tyr-Ser-Gln-Val-Leu-Phe-Lys-Gly-Gln-Gly-Cys-Pro-Ser-Thr-His-Val-Leu-Leu-Thr-His-Thr-Ile, as amino acids 59-80 of SEQ ID NO:1; and

Tyr-Gln-Thr-Lys-Val-Asn-Leu-Leu-Ser-Ala-Ile-Lys-Ser-Pro-Cys-Gln-Arg-Glu-Thr-Pro-Glu-Gly as amino acids 87-108 of SEQ ID NO:1.

10 24. A pharmaceutical composition, comprising an antibody according to claim 1, or fragment, region or pharmaceutically acceptable ester, ether, sulfate, carbonate, glucuronide or salt thereof, and a pharmaceutically acceptable carrier.

15 25. An isolated TNF polynucleotide, comprising a nucleotide sequence encoding an antibody according to claim 6, wherein said nucleotide sequence encodes at least one variable region in operable linkage with at least one constant region.

20 26. A polynucleotide according to claim 25, wherein said nucleotide sequence is selected from a genomic DNA sequence or a cDNA sequence.

27. A polynucleotide according to claim 25, wherein said polynucleotide is an expression vehicle.

25 28. A host transformed or transfected with the polynucleotide according to claim 25.

29. A host according to claim 28, wherein said host is a eukaryotic cell or a bacterial cell.

30. A host according to claim 29, wherein said host is mammalian cell.

31. A process for preparing an antibody, fragment or region according to claim 6, comprising:

5 (a) culturing a host according to claim 28 such that said antibody is expressed in recoverable amounts; and

(b) recovering said antibody, or a fragment or region thereof, from said host or culture.

10 32. A method for treating an animal having a pathology mediated by a TNF comprising administering to said animal a therapeutic amount of a pharmaceutical composition according to claim 24.^{PK2}

15 33. A method for treating an animal having a pathology mediated by a TNF comprising administering to said animal a therapeutic amount of a pharmaceutical composition according to claim 42.^{du PK2}

20 34. A method of removing from a sample a TNF α , a fragment thereof, or an immune complex containing said TNF α , the method comprising:

25 (a) contacting said sample to a device containing an antibody according to claim 1, or a fragment or region thereof, bound to a support, such that said TNF α , portion thereof or immune complex reversibly binds to said immobilized antibody, fragment or region to provide a bound TNF α , portion or immune complex; and

30 (b) recovering said bound TNF α , portion or immune complex from said bound antibody, fragment or region.

35. A method of removing from a sample a TNF, a fragment thereof, or an immune complex containing said TNF, the method comprising:

- 5 (a) contacting said sample to a device containing an antibody according to claim 6, or a fragment or region thereof, bound to a support, such that said TNF, portion thereof or immune complex reversibly binds to said immobilized antibody, fragment or region to
10 provide a bound TNF, portion or immune complex; and
- (b) recovering said bound TNF, portion or immune complex from said bound antibody, fragment or region.

15 36. A method of treating an animal subject suspected of having a pathology or condition associated with elevated levels of TNF in a body fluid, comprising:

- (a) removing said TNF from said body fluid using a method according to claim 34; and
20 (b) returning said body fluid to said animal.

37. A method of treating an animal suspected of having a pathology or condition associated with elevated levels of TNF in a body fluid, comprising:

- 25 (a) removing said TNF from said body fluid using a method according to claim 35; and
- (b) returning said body fluid to said animal.

38. An immunoassay method for detecting human TNF in a sample, comprising:

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- (a) contacting said sample with an antibody according to claim 1, or a fragment or region thereof; and
- (b) detecting the binding of the antibody to said TNF.

39. An immunoassay method for detecting human TNF in a sample, comprising:

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- (a) contacting said sample with an antibody according to claim 6, or a fragment or region thereof; and
- (b) detecting the binding of the antibody to said TNF.

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40. A method of treating an animal according to claim 32, wherein said pathology is selected from sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, a bacterial infection, a viral infection, a fungal infection, systemic lupus erythematosus, rheumatoid arthritis, alcohol-induced hepatitis, a chronic inflammatory pathology, a vascular inflammatory pathology, a graft-versus-host pathology, Kaisaki's pathology and a malignant pathology.

41. A method of treating an animal according to claim 33, wherein said pathology is selected from sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, a
5 bacterial infection, a viral infection, a fungal infection, systemic lupus erythematosus, rheumatoid arthritis, alcohol-induced hepatitis, a chronic inflammatory pathology, a vascular inflammatory pathology, a graft-versus-host pathology, Kaisaki's pathology and a
10 malignant pathology.

42. A pharmaceutical composition, comprising an antibody according to claim 6, or fragment, region or pharmaceutically acceptable ester, ether, sulfate, carbonate, glucuronide or salt thereof, and a
15 pharmaceutically acceptable carrier.

43. A polynucleotide according to claim 25, wherein said nucleotide sequence comprises a polynucleotide which encodes said at least one variable region or said at least one constant region, wherein at least a portion or
20 said polynucleotide hybridizes to at least a 15 base oligonucleotide complimentary to the sequence presented in Figure 17A (SEQ ID NO:2) or Figure 17B (SEQ ID NO:3).

44. A method according to claim 32, wherein said animal is a human.

25 45. A method according to claim 33, wherein said animal is a human.

46. A method according to claim 34, wherein said animal is a human. *Qeds cont.*

47. A method according to claim 37, wherein said animal is a human.

48. A method according to claim 40, wherein said pathology is rheumatoid arthritis.

5 49. A method according to claim 41, wherein said pathology is rheumatoid arthritis.

50. A method according to claim 48, wherein said pharmaceutical composition is administered in an amount of 0.1 to 50 mg/kg.

10 51. A method according to claim 49, wherein said pharmaceutical composition is administered in an amount of 0.1 to 50 mg/kg.